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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:
Bachovchin

Serial No: 09/628,225

Filed: July 28, 2000

For: Method of Regulating Glucose Metabolism, And Reagents Related Thereto

Attorney Docket No. TUU-P01-006

Art Unit: 1654

Examiner: J. Russel

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Declaration Under 37 CFR 1.131 of Daniel Drucker

Sir:

I, Daniel Drucker of Toronto, Canada, hereby declare as follows:

1. I am a co-inventor of the above-mentioned application which teaches and claims uses of dipeptidylpeptidase IV inhibitors for treatment of Type II diabetes, modifying GLP-1 metabolism, and modifying glucose metabolism, among other things.

2. The invention as described and claimed in the above-identified application was conceived prior to the publication of Balkan et al., Diabetologia, Suppl 40, A131 Abstract, which published in June of 1997.

3. In support of this, I attach as Exhibit A the text of an e-mail from me to coinventor Andrew Plaut memorializing our decision to administer Pro(boro)Pro in an oral glucose tolerance test (OGTT) in mice to confirm its effectiveness for treating Type II diabetes, modifying GLP-1 metabolism, or modifying glucose metabolism. See Exhibit B for the structure of Pro(boro)Pro. This compound is a potent dipeptidylpeptidase IV inhibitor. The OGTT in this case measures the efficacy of Pro(boro)Pro in reducing blood glucose levels when administered to mice that had ingested a measured amount of glucose after a period of fasting. I sent the e-mail of Exhibit A prior to June 1997.

4. After sending the e-mail of Exhibit A, the OGTT experiments were planned to be carried out using mice having normally functioning GLP-1 processes (GLP-1 +/+ mice) and GLP-1 knockout mice (GLP-1 -/- mice). (See Exhibit C) During the four months after sending the e-mail of Exhibit A, we received a batch of Pro(boro)Pro from co-inventor Dr. Andrew Plaut and ordered the GLP-1+/+ mice, the GLP-1 -/- mice, and other materials required to carry out the experiment. The OGTT experiments were commenced within four months of having sent the e-mail of Exhibit A, and results were obtained within two months after that. The results showed that Pro(boro)Pro improves glucose tolerance in both mice models. (See Exhibit D)

5. Type II diabetes is characterized by glucose intolerance. The mice used in our experiments are reasonable models of glucose intolerance.

6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title XVIII of the United States Code and that willful false statements may jeopardize the validity of this Application for Patent or any patent issuing thereon.

Daniel Drucker

Dated: April 12 [illegible] Signature: [illegible]

Exhibit A

From: IN%"d.drucker@utoronto.ca" 12-MAY-1997
Hi Anfrew,

I may have met a very distant cousin of yours in Baltimore (Allergy and Immunology, now at the NIH?) this weekend.

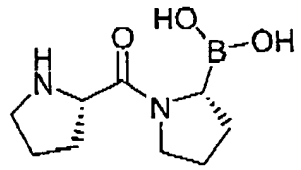
On perusing the papers you sent me, the best experiments would be those we could carry out in rats. This is because a)rats have higher levels of DP-IV than mice and b) we could get more blood from the rats for HPLC/RIA experiments to show the precise molecular forms of peptides in the presence and absence of inhibitors. Any data on the use of the inhibitors in rats from your group? I am extrapolating from the []s of inhibitor used in mice as follows, so correct me if I make a mistake: The [] of inhibitor that is reasonably effective is 1 ug/kg sc twice a day.

If we do rats, then this would be ~250 ug twice a day, x 10 rats for example, x 10 days (for the bowel growth/GLP-2 effects). Do you have these amounts of inhibitor available? If so, we could try and get these studies done in the next few months. We could also do some more acute effects with OGTT and GLP-1 that would be short-term studies that wouldn't require much inhibitor. Let me know if the amount of material required is a problem. DD

**********Daniel

Drucker M.D. 416-340-4125 voice 416-978-4108 Fax
Director, Endocrine Division
Department of Medicine
University of Toronto
The Toronto Hospital
200 Elizabeth Street CCRW3-838
Toronto, Ontario Canada M5G 2C4

Exhibit B



Pro(boro)Pro

Exhibit C

dissolve at pH 2 + store at 4°C or freeze

Pro(boro)Pro Experiments

Previous Experiments

- Involvement of DPIV in an *in vivo* immune response AND DPIV activity in serum and on lymphocytes of MRL/Mp-*lpr/lpr* mice correlates with disease onset (both Kubota *et al.*)

•Pro(boro)Pro conc. (ug):	20	40	80	160*
% DPIV activity in plasma:	9.6%	6.7%	3.8%	1.9%

[1 hour following sub. cu. injection (BALB/c mice)]

*No toxicity observed at high doses.

•Dissolve Pro(boro)Pro in saline and filter sterilize.

•Repeated injections of 10 ug Pro(boro)Pro at 12 h intervals maintained *in vivo* DPIV activity at less than 30% of the normal level. (Measured two hours after the fourth injection of 10 ug Pro(boro)Pro at 12h intervals).

•Assay for DPIV: 10 ul plasma incubated with fluorogenic substrate Ala-Pro-AFC (Enzyme Systems Products, Dublin, CA) in cuvette of a Luminescense Spectrometer.

•Pro(boro)Pro t1/2 = 1.5h

•Approximately 50% of normal DPIV activity is observed 23h following the last injection.

Experiment 1 (Week of Sept. 15)

•8 male +/+ mice, 6-8 weeks old

•4 receive 160 ug subcutaneous Pro(boro)Pro/saline injection, 1h prior to OGTT

•4 receive subcutaneous saline injection 1h prior to OGTT.

•Obtain plasma sample at between 20-30 min time point of OGTT to measure DPIV activity and/or plasma insulin levels. GLP-1 levels could also be measured by HPLC or RIA if total bloods were obtained by cardiac puncture following the 120 minute time point (assuming the RIA Ab is able to distinguish between active 7-36 and inactive 9-36 GLP-1)

•Expected Results: Should observe decreased blood glucose excursion in mice receiving Pro(boro)Pro injection due to increased GLP-1/ GIP insulinotropic action. Plasma insulin levels are expected to be increased in mice receiving Pro(boro)Pro, DPIV plasma activity should be drastically reduced, and plasma concentrations of active GLP-1 should be elevated in mice receiving Pro(boro)Pro.

•If glucose tolerance is not significantly improved in mice receiving a single dose of Pro(boro)Pro, administer four consecutive doses of Pro(boro)Pro at 12h intervals, and commence an OGTT 1h following the last injection.

8 +/+

4 saline

1hr

OGTT

probably won't see much of an effect when only endogenous GLP-1 is present.

Experiment 2

•Repeat above experiment with GLP-1R -/- mice. Expect glucose tolerance to be improved in -/- receiving Pro(boro)Pro due to increased GIP action, although not as dramatically as +/+ because GLP-1 signaling is absent. Although there is evidence that GIP sensitivity is enhanced in the -/- mice, apparently in an effort to compensate for the absence of GLP-1, the GLP-1R -/- mice are still glucose intolerant, suggesting that increased GIP sensitivity is insufficient to maintain normal glucose control in these mice. Thus, further increases in GIP action in the -/- mice treated with DPIV-inhibitor is not expected to decrease glucose excursion as dramatically as in +/+ mice treated with Pro(boro)Pro.

Experiment 3

•8 +/+ male mice

•4 receive both Pro(boro)Pro (1h prior to OGTT) and GLP-1 (5 min prior to OGTT) injections

•4 receive a saline injection 1h prior to the OGTT, and a GLP-1 injection 5 minutes prior to OGTT.

•Expected result: Pro(boro)Pro coadministered with GLP-1 should enhance the effectiveness of GLP-1 in suppressing glucose excursion following oral glucose administration. This supports the idea that an analogue of GLP-1 resistant to degradation by DPIV would be more effective in the treatment of NIDDM.

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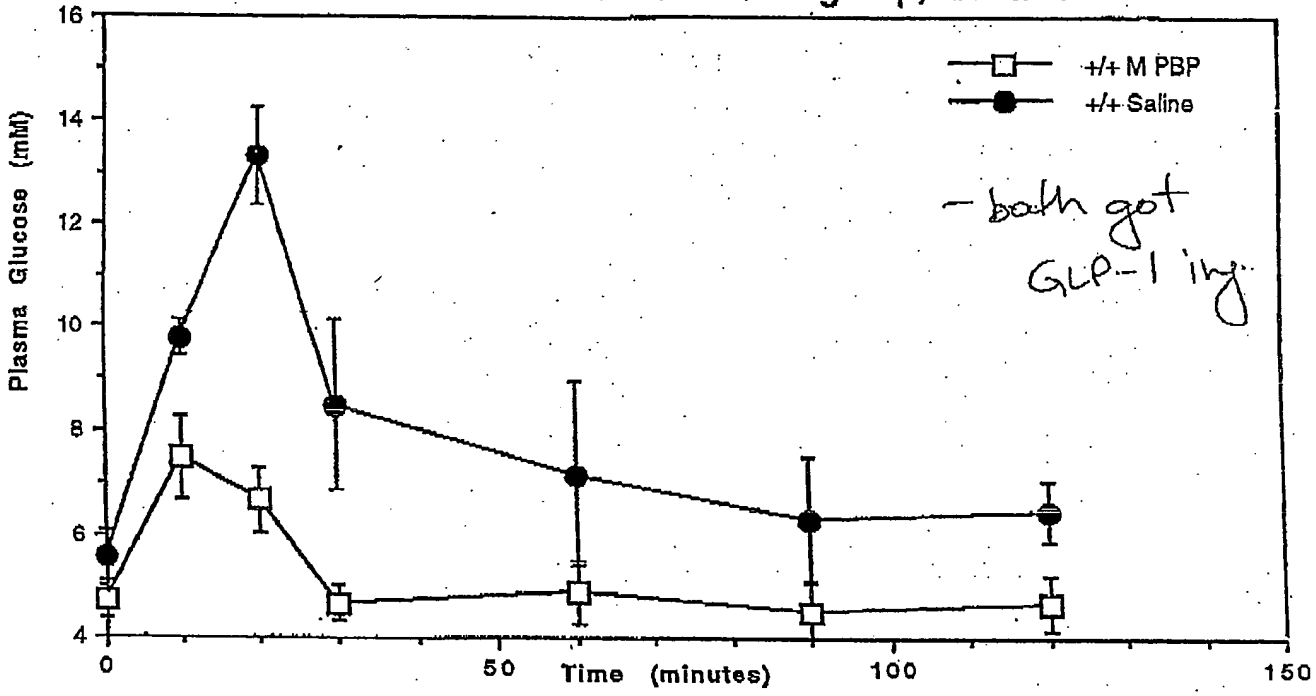
FIRST - injected PBP 1 hr. prior to OGTT, then GLP-1 5 mins. prior to OGTT
- gave GLP-1 because we didn't think we'd see effect w/ endogenous GIP + GLP-1 only

+/+

PBP-1

+/+ males, receiving either saline or 150ug Pro(boro)pro 1 hr prior to oral glucose challenge AND 8 ug GLP-1 5 mins prior to glucose load (6 weeks old as of)

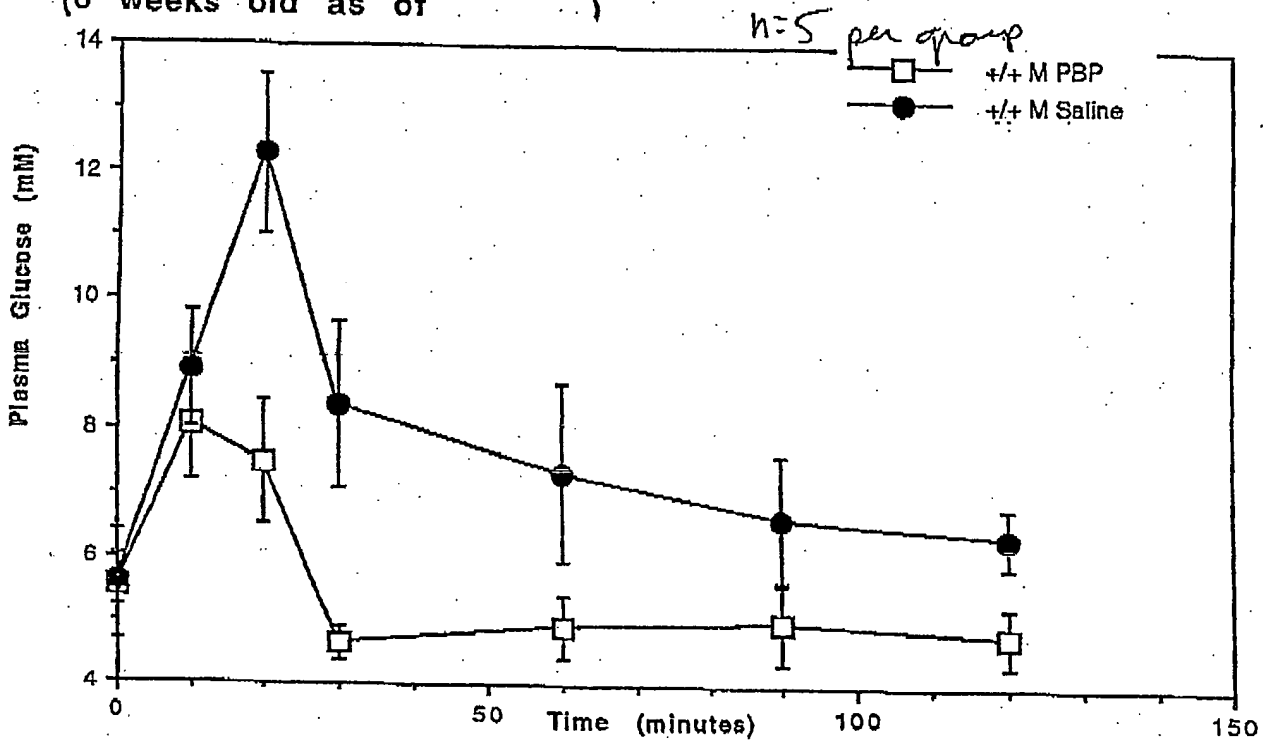
***Data from one mouse removed from each group, so n=4**



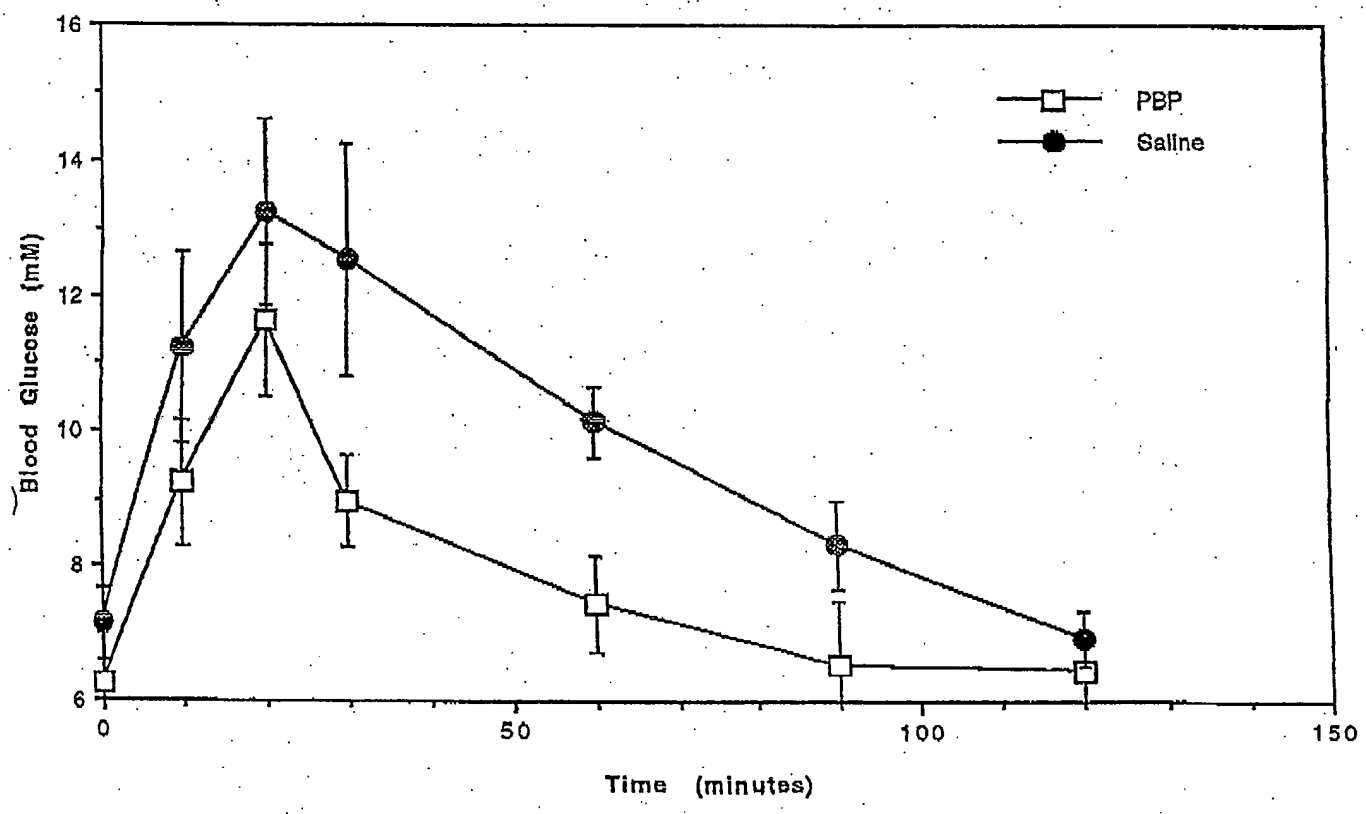
- both got GLP-1 inj.

PBP-1

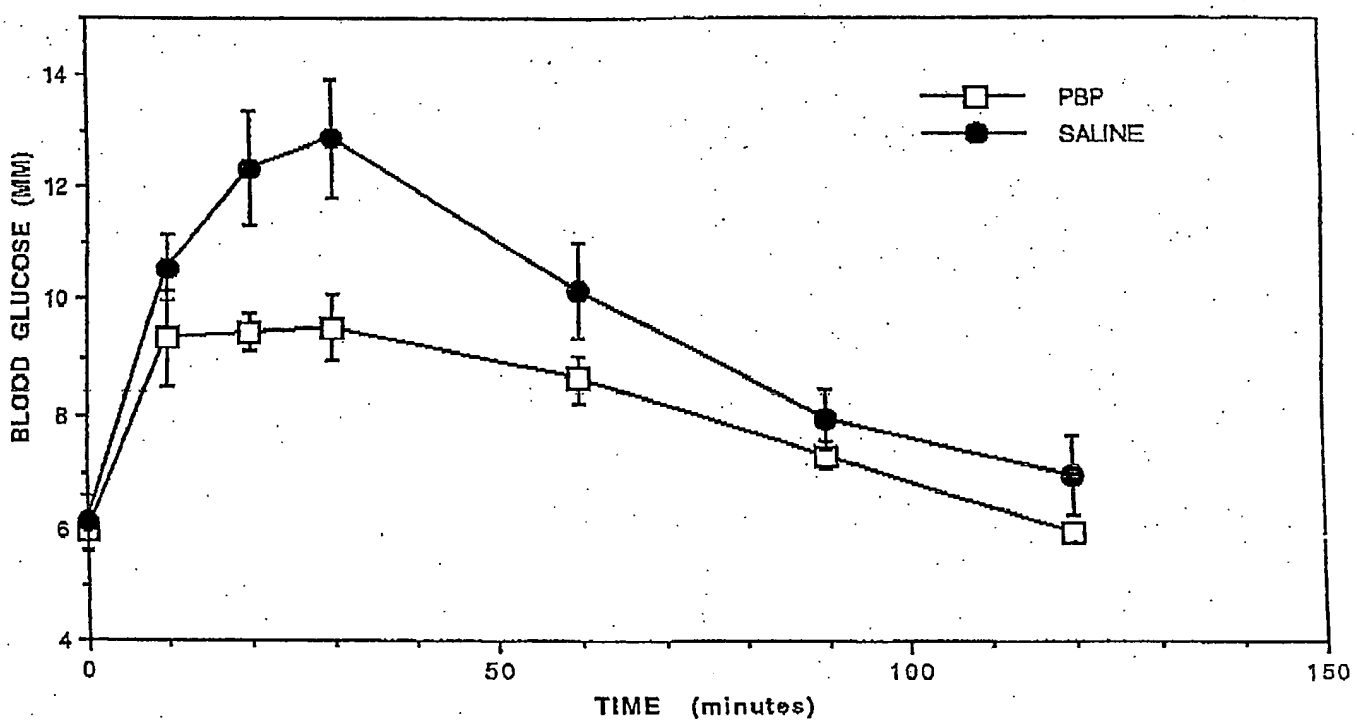
+/+ males, receiving either saline or 150ug Pro(boro)pro 1 hr prior to oral glucose challenge AND 8 ug GLP-1 5 mins prior to glucose loac (6 weeks old as of)



PBP-2 +/+ Males (6 weeks of age as of) injected with either 150ug Pro(boro)pro or saline 1 hour prior to an OGTT



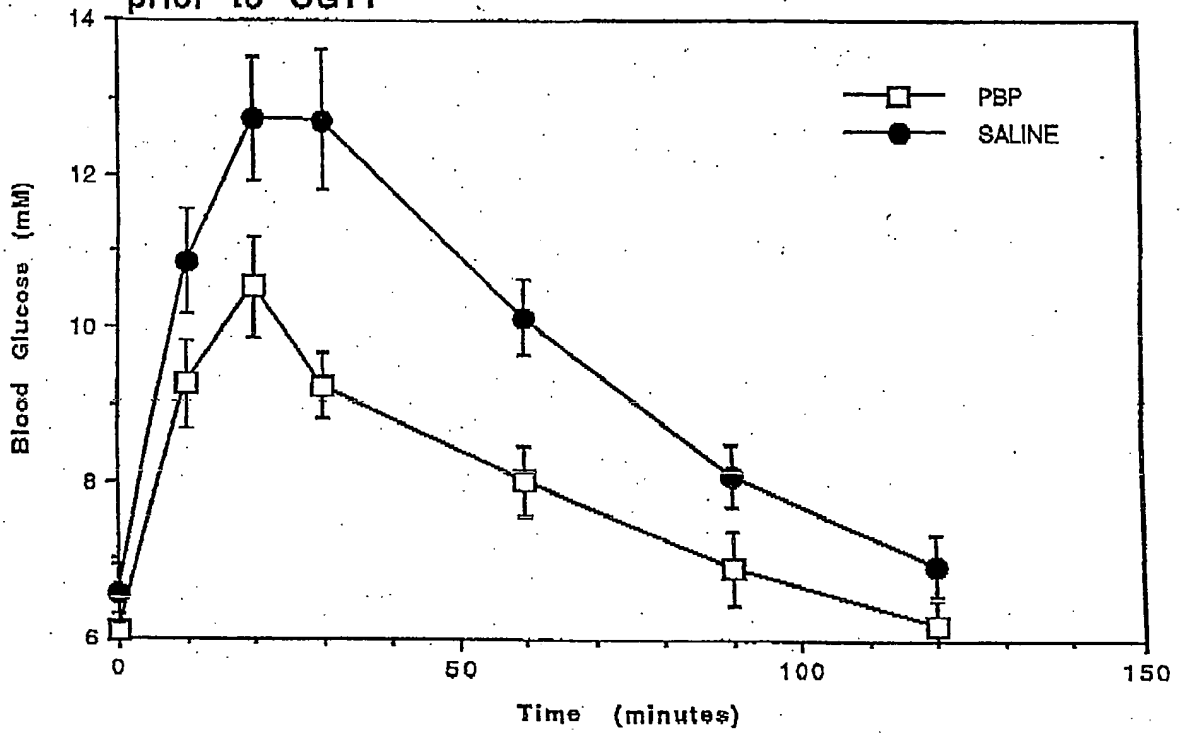
PbP-2 Repeat XX Male mice (6 weeks old as of) were injected with either 150ug Pro(boror)pro OR saline 1 hr prior to OGTT



Then - just PBP or saline

+/+

PBP-2 Combined Data x/x Male mice (6 weeks old as of . .) were injected with either 150ug Pro(boro)pro OR saline 1 hr prior to OGTT



+/+ ♂

250ul Saline + 250ul PBP

IP injection

PBP-3 +/+ Females injected with 150ug Pro(boro)pro OR saline twice daily as of (9 injections) 7-8 weeks old as of

250ul